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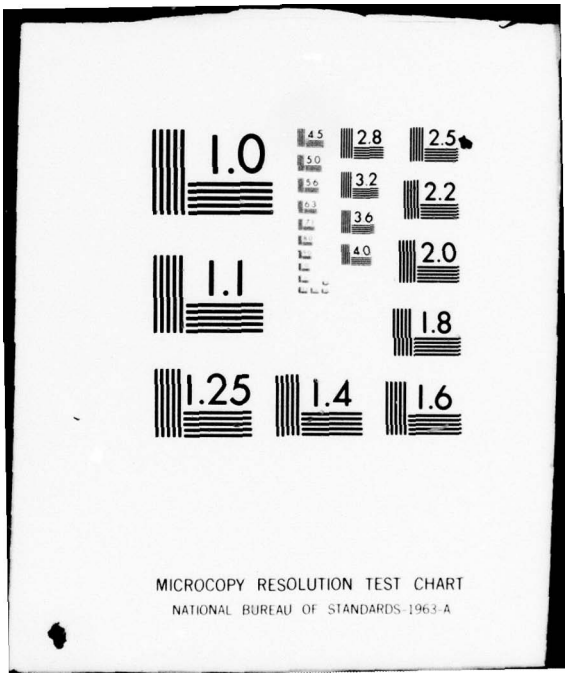
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KOREAN HEMORRHAGIC FEVER

Final Report

HO WANG LEE, M. D.

March 1978

Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Epidemic hemorrhagic fever was recognized for the first time in Korea in 1951 and since that time it has been known as Korean hemorrhagic fever (KHF). Diseases similar to KHF have been known in Manchuria, the Soviet Union, Scandinavia, Eurasia and Japan.

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

Many scientists have been conducting research for the detection of the etiological agent of KHF and similar diseases but without any particular achievement until 1975. However, in 1976 Lee and Lee found an antigen from Apodemus agrarius which reacts specifically to convalescent sera of KHF patients by fluorescent antibody techniques (FAT) and named it Korea antigen.

cont

The purpose of the research was (1) to isolate the etiologic agent of KHF, (2) to propagate the etiologic agent of KHF in animals; and (3) to study the serologic relationship between KHF agent and other acute hemorrhagic fevers of the world.

The etiologic agent of KHF was isolated from lung tissues of Apodemus rodents and from acute phase sera of patients by FAT. The agent was successfully propagated in Apodemus agrarius through 26 passages but could not be cultivated in cell cultures nor in laboratory animals. Diagnostic increases in immunofluorescent antibodies occurred in 113 of 116 severe and 11 of 34 milder cases clinically suspected of having KHF. Antibodies were present during the first week of symptoms, reached a peak level at the end of the second week and persisted for up to 14 years. Antibody responses to KHF agent after subcutaneous inoculation into rabbits were demonstrated. Convalescent sera from persons suffering a similar disease in the Soviet Union and in Japan were positive for antibodies but antisera for the arenaviruses, Marburg and Ebola were negative.

LEVEL II

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SUMMARY

Ninety-three strains of the etiologic agent of KHF which reacted specifically with the convalescent sera of KHF patients were isolated from lung tissues of Apodemus agrarius coreae rodents and also the same agents were isolated from acute sera of two patients in adult Apodemus. Virus-like particles (spherical, about 50 nm in diameter, in crystalline array) were observed at cytoplasms of pulmonary tissues from 5 wild Apodemus and proved to be KHF agent positive by FAT.

The agent was successfully propagated in Apodemus through 26 passages. Experimentally inoculated mice developed specific fluorescent antigen in lung, kidney, liver, parotid glands and bladder.

Diagnostic increases in immunofluorescent antibodies occurred in 113 of 116 severe and 11 of 34 milder cases clinically suspected of having KHF. Antibodies were present during the first week of symptoms, reached a peak at the end of the second week and persisted for up to 14 years.

Distribution of IF antibodies to KHF agent in the sera of residents of non-endemic and endemic areas are 1.0% and 3.8%, respectively.

Convalescent sera from patients in the Soviet Union with hemorrhagic fever with renal syndrome and in Japan with epidemic hemorrhagic fever were positive for antibodies.

No fluorescence was observed when infected Apodemus lung tissue was reacted with antisera to Marburg, Ebola and several arenaviruses.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animals, Resources, National Academy of Sciences-National Research Council.

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INTRODUCTION

Epidemic hemorrhagic fever was recognized for the first time in Korea in 1951 among United Nation troops (1). Since that time it has been known as Korean Hemorrhagic Fever (KHF) and has remained endemic near the Demilitarized Zone between North and South Korea. In recent years the disease has invaded the southern parts of the Korean peninsula and 100 to 800 hospitalized cases are clinically diagnosed each year (Table 1).

Diseases similar to KHF have been described by Japanese from Manchuria (2.3.4), from the Soviet Union (5.6), from Scandinavia (7.8), from several countries in Eastern Europe (9) and recently from Japan (10). In the early 1940's, Japanese and Russians successfully reproduced hemorrhagic fever by injection of urine and sera of patients in the acute stage into volunteers (3.4.5). Filtered sera of the patients also produced clinical symptoms, so this disease has been suspected as being of viral origin. Injection of a suspension of Trombicula mite obtained from Apodemus agrarius into humans caused hemorrhagic fever, and mites have been suspected as its reservoir (11).

Many attempts have been made to isolate the causative agent of KHF and clinically similar diseases. A Soviet report of cultivation of an agent in cell cultures from patients with hemorrhagic nephroso-nephritis (12), has not been confirmed.

In 1976 Lee and Lee (13) succeeded in demonstrating an antigen in the lungs of the striped field mouse, Apodemus agrarius, which produced on immunofluorescent reaction with sera from patients convalescent from KHF. Lee and Lee named it Korea antigen. Very recently, Lee et al (14) have reported that this antigen is the etiologic agent of KHF and is produced by a replicating microbe.

This report is the results of the project on isolation of KHF agent and the serologic relationship between KHF and other viral hemorrhagic fevers.

MATERIALS AND METHODS

Survey areas

Surveys were carried out in civilian farm villages where many cases of KHF have been reported each year. Six areas, namely Yunchun, Pochun, Songnaeri, Jihengri, Chunsuri and Kasangri of Kyungido, Korea were surveyed.

Collection, identification and processing of rodents

Rodents were captured from fields, uncultivated scrub vegetation and near farm dwellings using live traps. Traps were set in the late afternoon and examined at midnight and at dawn. Animals were transported live to the laboratory in Seoul and identified according to the taxonomic scheme of Won (15). Blood samples were obtained by cardiac puncture under chloroform anesthesia and the animals were autopsied.

Cervical lymph nodes, spleen, lungs, liver and kidneys were removed and weighed. Portions of each organ were triturated in BSS, pH 7.4 containing 1 percent bovine plasma albumin (BSSA) for virus isolation attempts, and the remainder was stored at -70° C.

Apodemus agrarius rodents for laboratory studies

Apodemus agrarius coreae were obtained at Chullanamdo, Chindo Island and Apodemus agrarius jejudoica were trapped on Jeju Island. Neither area has ever registered cases of KHF. These animals weighed from 20-50 g.

Strains of KHF agent employed

All experimental and diagnostic work was done with lung tissues originating from 6 naturally infected Apodemus agrarius coreae.

Designations and source data are:

1. #75-191, Pochun 10-06-75
2. #75-206, Songnaeri 10-27-75
3. #76-66, Yunchun 5-06-76
4. #76-100, Songnaeri 6-02-76
5. #76-118, Songnaeri 6-23-76
6. #76-236, Songnaeri 10-07-76

For fluorescent antibody tests frozen sections of lung tissue were cut at 4 μ, air dried on glass slides, fixed for 7 minutes in acetone and stored at -70° C until needed.

To titrate the infectious agent 10 percent lung suspensions were prepared in BSSA, clarified at 2,000 g for 20' and supernatants were used to inoculate cell cultures and animals.

Titration endpoints in Apodemus agrarius rodents were calculated 20 days later by the Reed-Muench method.

Fluorescent antibody techniques (FAT)

Both direct and indirect (IFA) procedures used methods described elsewhere (13.14). FITC-conjugated polyvalent immunoglobulins of goat origin prepared against human, guinea pig, mouse, rat and rabbit immunoglobulins were purchased from Hyland Co., Calif.

Electron microscopic (EM) examination

Among the glutaraldehyde-fixed lung tissues stored at 4° C, 6 tissues which most clearly visualized specific fluorescence were selected from the tissues in which the presence of KHF agent were confirmed by FAT. The methods of Watson (16) and Reynolds (17) were employed for staining unpassed Apodemus tissue sections and observed under electron microscope (Hitachi HS-7S 50 KV). Apodemus agrarius coreae which proved to be normal by FAT were used as controls.

KHF patients and normal human sera

An initial blood sample was taken as soon as the patient was hospitalized and blood samplings were done at regular intervals during the course of illness. Sera of U. S. personnel in Japan who had never been to Korea were obtained from Col. J. D. Marshall and used as controls.

Rabbits for antibody production

White New Zealand rabbits weighing 2-3 kg were used for production of specific antibodies against KHF agent. 8,000 Apodemus ID_{50} of KHF agent, 76-118, 7th passage in Apodemus, was inoculated into a rabbit subcutaneously and the antibody titers of sera were measured by IFA technique at certain intervals after inoculation.

RESULTS

1. Isolation of etiologic agent of Korean Hemorrhagic Fever

Frozen tissues from 921 rodents captured during 1974-1977, in districts where cases of human KHF had occurred, were examined by the IFA technique. Fine granular fluorescence was observed in sections of lung (Figure 1) and renal tissue obtained from Apodemus agrarius coreae rodents when stained with convalescent serum from KHF case 74-74. This reaction was present to a serum dilution of 1:2,048, whereas serum obtained from the same patient on the third day of clinical illness was positive only to a dilution of 1:16. Sera from several persons never resident in Asia or Europe failed to react. Ninety-three of 696 Apodemus agrarius (13.4 percent) were positive but none of 225 specimens from 7 other species gave specific staining. The seasonal distribution of rodent captures and positive IFA reactions is depicted in Table 2.

Acute and convalescent sera samples were obtained from more than 100 hospitalized cases of suspected KHF during 1976. Only 11 of those which were shown subsequently to have diagnostic increases in IFA titer had no antibody in their first serum specimen. The interval from onset of symptoms to collection of these sera was 2-6 days. Sera were stored at -70° C for 1-12 weeks, then inoculated by intrapulmonary and subcutaneous routes into adult Apodemus, 2 cases were positive as shown in Table 3.

2. Electron microscopic findings of infected lungs of Apodemus agrarius rodents with KHF agent

Limited studies have been completed and these are definitely preliminary results. Five pulmonary tissues having the most antigen among those proved to be antigen-positive by FAT were screened by EM. It was observed that all of them held the same types of virus-like particles (Figure 2). These particles were connected with minute rod-shaped aggregates, forming crystalline bodies and were situated at cytoplasm. The particle aggregates formed their package and the cell containing the aggregate was alveolar epithelium. Each particle looked like a spherical form, about 50 nm in diameter, with an electron dense core.

3. Propagation of KHF agent in Apodemus agrarius rodents

Multiplication and infection of KHF agent in Apodemus after inoculation were examined. A 5% lung suspension of strain 76-118, 1st passage Apodemus tissue was inoculated by intrapulmonary and subcutaneous routes into 50 wild-caught Apodemus rodents. Lung tissues were examined from animals sacrificed at intervals from 9 to 69 days (Table 4). Because the mice used for this experiment were from the Korean mainland, we were unable to exclude the possibility that some of them were naturally infected. Nevertheless, positive reactions were seen significantly more often between 13-27 days after inoculation (67%) than during earlier (25%) or later periods (30%). These data suggest that the agent multiplied in the inoculated Apodemus, and that infection, as measured by immunofluorescence, was not a lifelong chronic phenomenon.

Distribution of KHF agent in Apodemus inoculated different routes is shown in Table 5. Intrapulmonary and subcutaneous inoculation were superior to intraperitoneal and nasal-oral routes and maximum fluorescence was observed in lungs at 20 days of incubation. No antigen was ever detected in spleen. As indicated in Table 6, similar variation in distribution and intensity of staining was observed in tissues of wild Apodemus captured during 1976. Neither wild-caught nor experimentally inoculated Apodemus ever displayed overt signs of clinical disease.

Serial passage of infected lung tissue suspension was made in Apodemus. As shown in Table 7, infection of all mice was not achieved until the 7th passage. By this time, the material contained $10^{4.2}$ 50% Apodemus IFA infectious units.

4. KHF-specific antibody responses in humans and animals

Most KHF patients had serum antibodies against the Apodemus agent by the end of the first week of symptoms. Nevertheless, increases in antibody titer in paired acute and convalescent sera were detected in 113 of 116 cases which displayed the classical clinical phases of fever and toxemia, hypotension, and diuresis (Table 8). Among 34 cases not having hypotensive or diuretic manifestations, only 11 were diagnosed as KHF by the IFA test.

The evolution of IFA antibodies during the 60-day period from disease onset is shown in Figure 3. Highest titers were observed at 2-3 weeks, followed by a slow decline.

Antibodies to the Apodemus agent following clinical KHF persisted for several years. Measurements made in one patient during a 7-year interval are depicted in Figure 4. Antibodies also were present in each of 13 sera samples obtained from KHF patients 3-14 years after acute illness. The geometric mean titer of these sera was 1:147.

Surveys for antibodies to the KHF agent were carried out using serum specimens obtained from persons resident in Seoul and in the endemic area.

As may be seen in Table 8, antibody prevalence among adult blood donors, out-patients and Korean soldiers was about 1 percent but from residents of endemic areas, 3.8 percent.

Immunofluorescent antibody responses to KHF agent after subcutaneous inoculation into rabbits are shown in Figure 5. All 5 rabbits received 8,000 Apodemus ID_{50} of KHF agent 76-118, 7th passage. They started to produce antibodies at 7 days, reached maximum levels at 14 days and then levels declined slowly by 60 days. The range of antibody titers at 14 days was between 256 to 8,192 and the pattern of antibody curve was almost the same as in humans. Anamnestic antibody responses to KHF agent after secondary inoculation were also observed and maximum titers were reached 2 days after booster injection (Figure 5).

5. Serologic relationship between KHF and other viral hemorrhagic fevers

To date only limited studies have been completed. Antisera for the following viruses obtained from man, monkey or guinea pig were tested by the IFA technique with negative results: Lassa, Machupo, lymphocytic choriomeningitis, Pichinde and Tacaribe of the arenavirus group, Marburg and Ebola (Table 9).

In contrast, definite relationships between KHF and hemorrhagic fever with renal syndrome in the Soviet Union and with epidemic hemorrhagic fever in Japan were established.

Four sera from Russian patients were correctly distinguished from four others obtained from residents of New York State. These sera were kindly supplied under code by Dr. Jordi Casals of the Yale Arbovirus Research Unit. 18 of 20 sera from patients with epidemic hemorrhagic fever in Japan about 10 years ago (10) were positive against KHF agent (18).

DISCUSSION

These observations, although definitely preliminary, provide substantial evidence that the causative agent of KHF has been isolated from wild rodent Apodemus agrarius coreae and from the blood of KHF patients. The important data may be summerized as follows: 1) Detection of antigen by IFA in lung and other tissues from Apodemus but not other species of rodents captured in KHF endemic areas. 2) Demonstration of the antigen in Apodemus lungs after serial passages representing dilution of the starting material more than 10^{-63}. 3) Failure to find IFA antigen in inoculated Apodemus for a period of 9 days followed by an increasing fraction of positive animals in the next two weeks.

Further support for the specificity of the association between agent and human disease included diagnostic increases in IFA titers (several of which were confirmed with blocking tests) in sera from nearly all of the large series of "typical" KHF cases, the detection of infection in a smaller fraction

of "atypical" mild cases, the low prevalence of antibodies in sera from urban Korean residents, and the identification of coded sera from persons surviving a similar clinical infection in the Soviet Union and Japan. These data considerably extend the original work of Lee and Lee (13) who reported on the relationship between Apodemus antigen and antibodies in KHF patients. The nature of the agent isolated in these studies awaits definitive characterization.

All attempts to establish the KHF agent in hosts other than Apodemus agrarius have been unsuccessful. Various species of laboratory animals, as well as several types of cell cultures, all failed to evidence specific IFA staining when inoculated with KHF agent. Animals tested included suckling white mice and weaned mice, hamsters, guinea pigs, rats and rabbits. Continuous cell lines employed were African green monkey kidney (Vero), dog embryo (R-1247), porcine kidney (PS), human embryo lung (WI-38), mink kidney (Mu 1 Lu), and Chinese hamster lung (Dede). Primary cell cultures prepared from human embryo kidney, rhesus monkey kidney, duck, and chicken embryo, rat liver and human embryonic liver also failed to develop cytopathic effect or specific immunofluorescence when inoculated with infectious Apodemus lung suspension. A host system which can be readily adapted to manipulation in the laboratory is needed and is being sought. Apodemus agrarius has never been colonized.

In the interim, animals of the subspecies jejudoica, which are apparently not naturally exposed to infection, will be employed in work designed to determine if the new agent is in fact a virus, whether it is related to any known agent, and whether or not ectoparasites of Apodemus agrarius are potential biological vectors of the pathogen.

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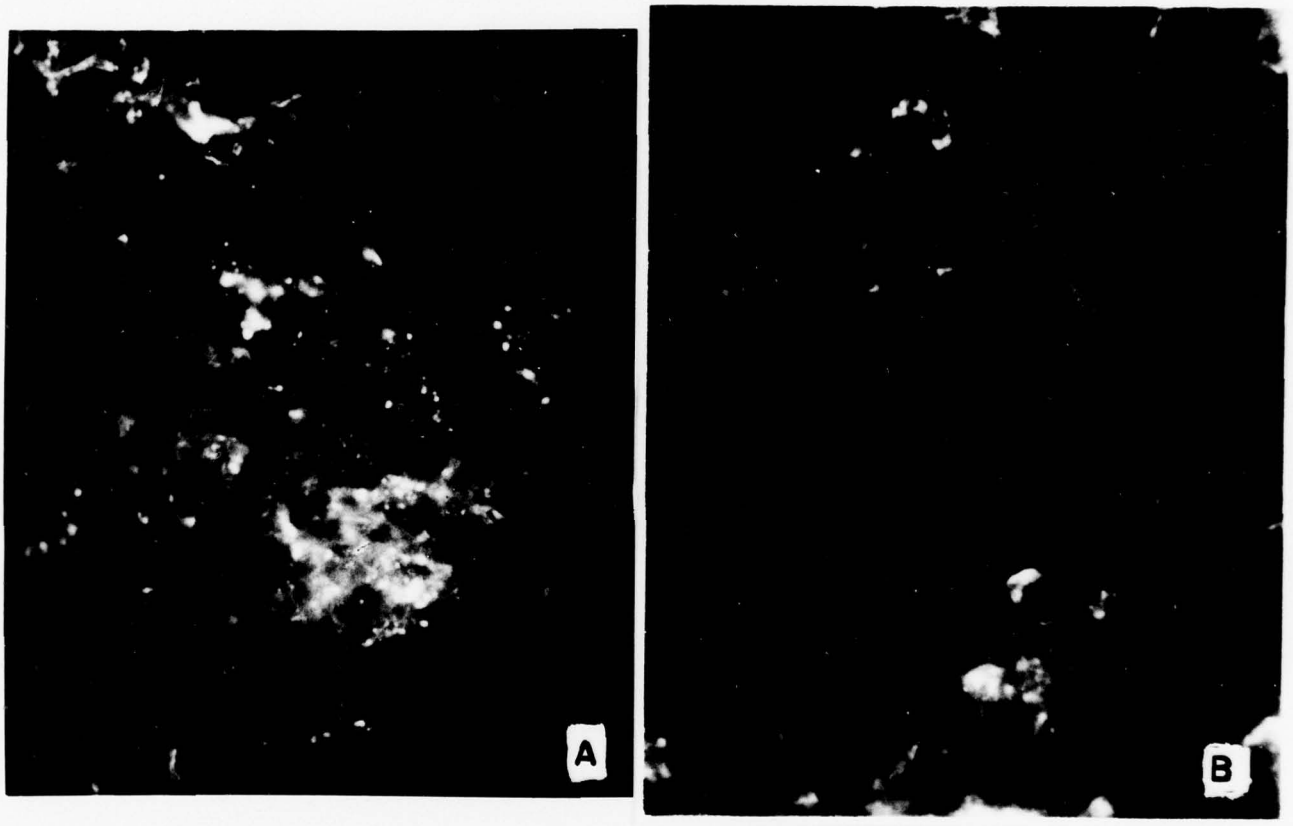

Fig. 1. Fluorescent antigen to KHF agent in lung tissues of Apodemus agrarius coreae. Antiserum used was from convalescent aptient 75-15-2. Indirect staining method, 400X.

A. Specific punctuate antigen inclusions in infected rodent.
B. Non-specific diffuse fluorescence in uninfected rodent.

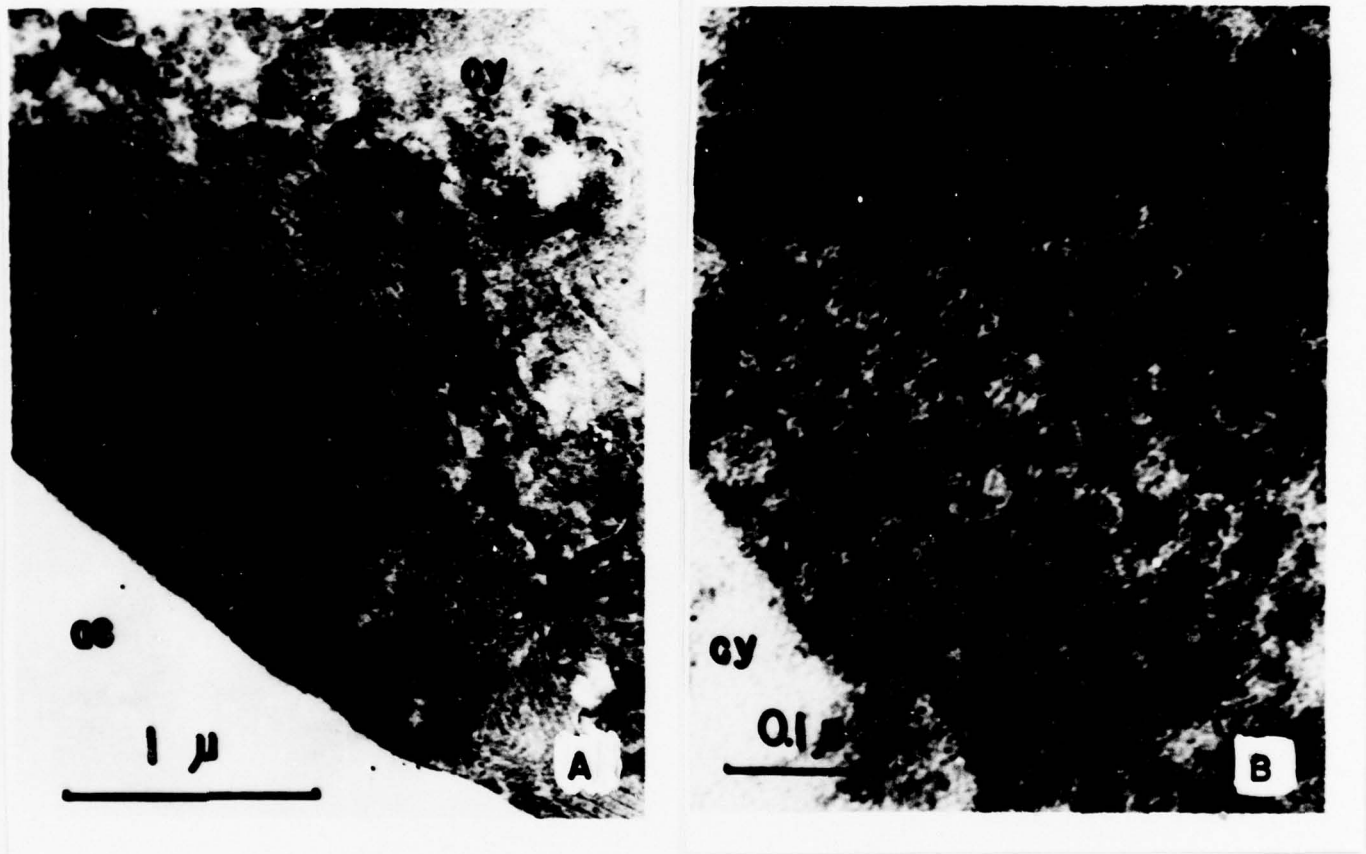

Fig. 2. Electron micrographs of KHF agent in lung tissues of Apodemus agrarius coreae.

A. Virus-like particles observed in ultrathin section of lung tissue.
B. Virus-like particles in crystalline array, round and about 50 nm diameter.

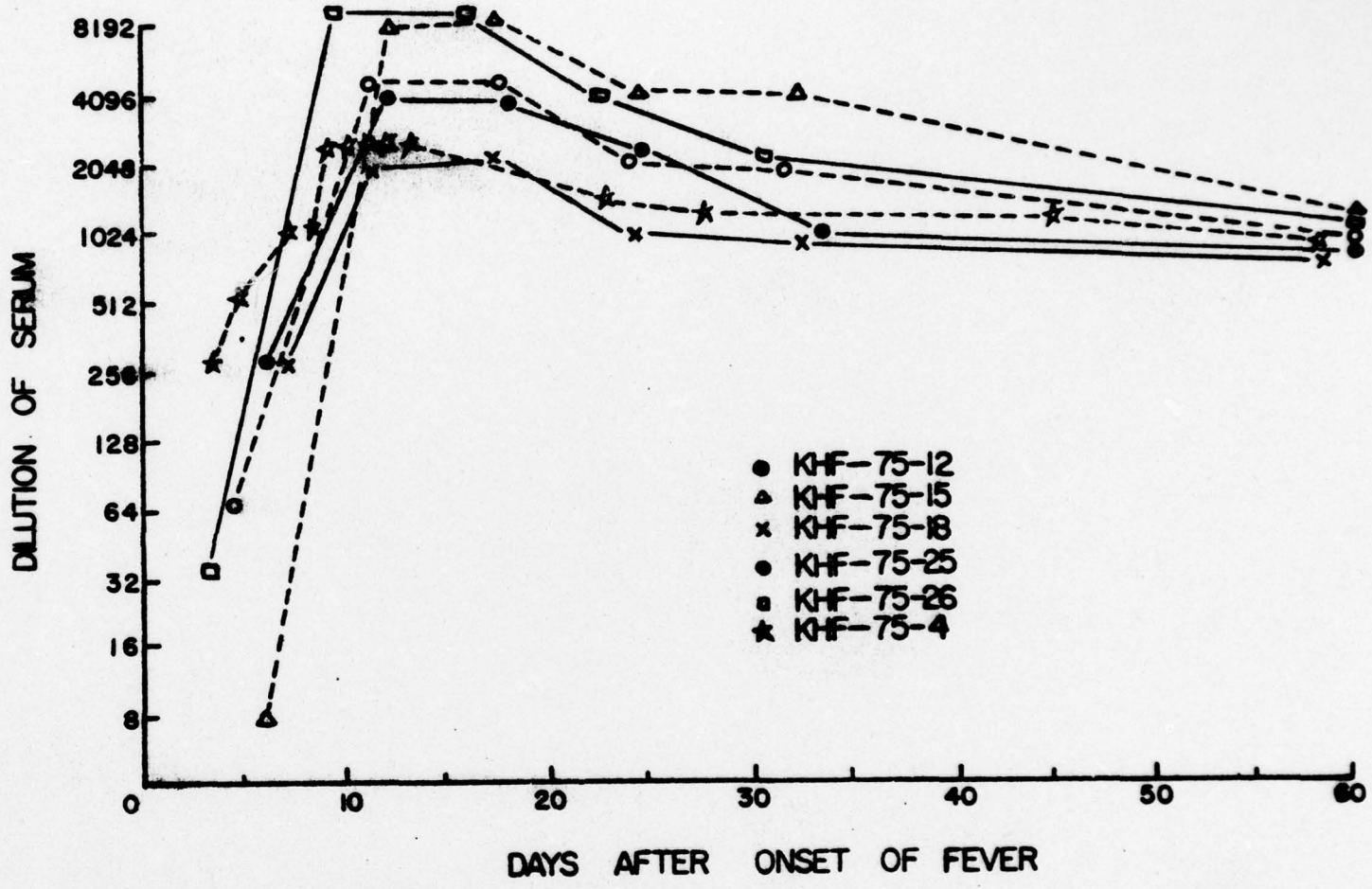

FIG. 3. IMMUNOFLUORESCENT ANTIBODY TITERS OF KHF PATIENTS TO KHF AGENT

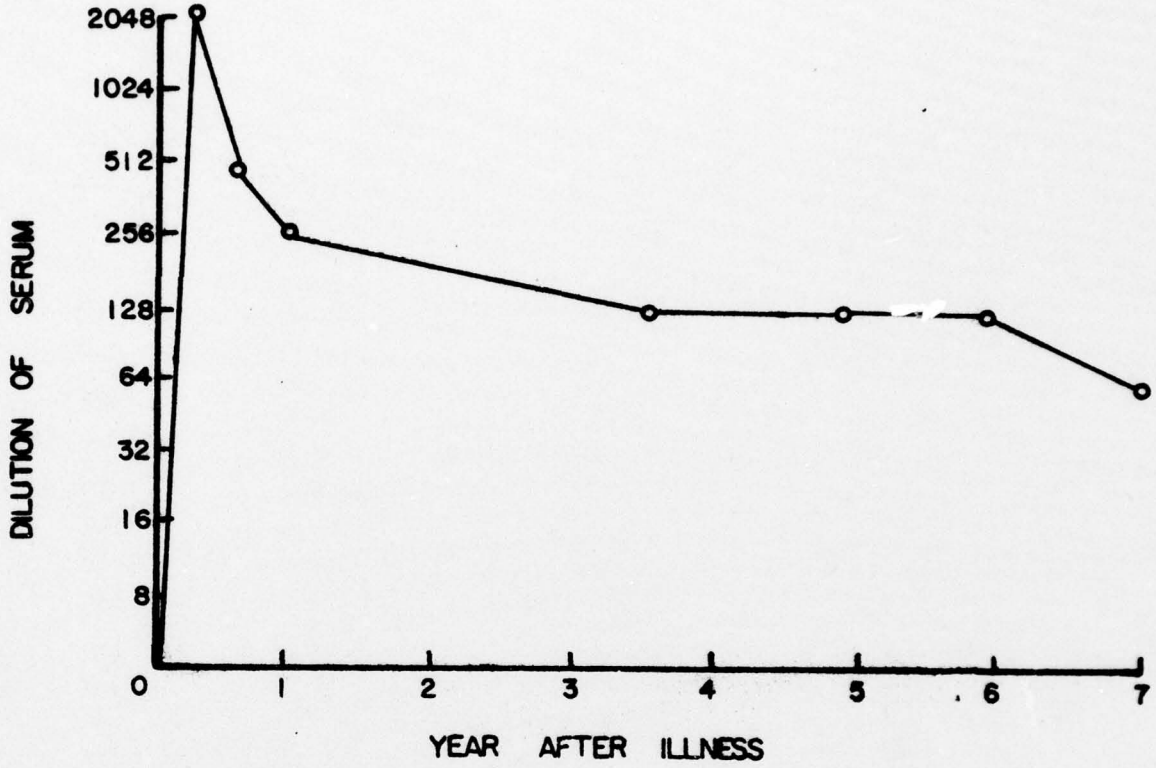

FIG. 4. PERSISTENCE OF IMMUNOFLUORESCENT ANTIBODIES TO KHF AGENT IN SERA OF A PATIENT WITH HEMORRHAGIC FEVER

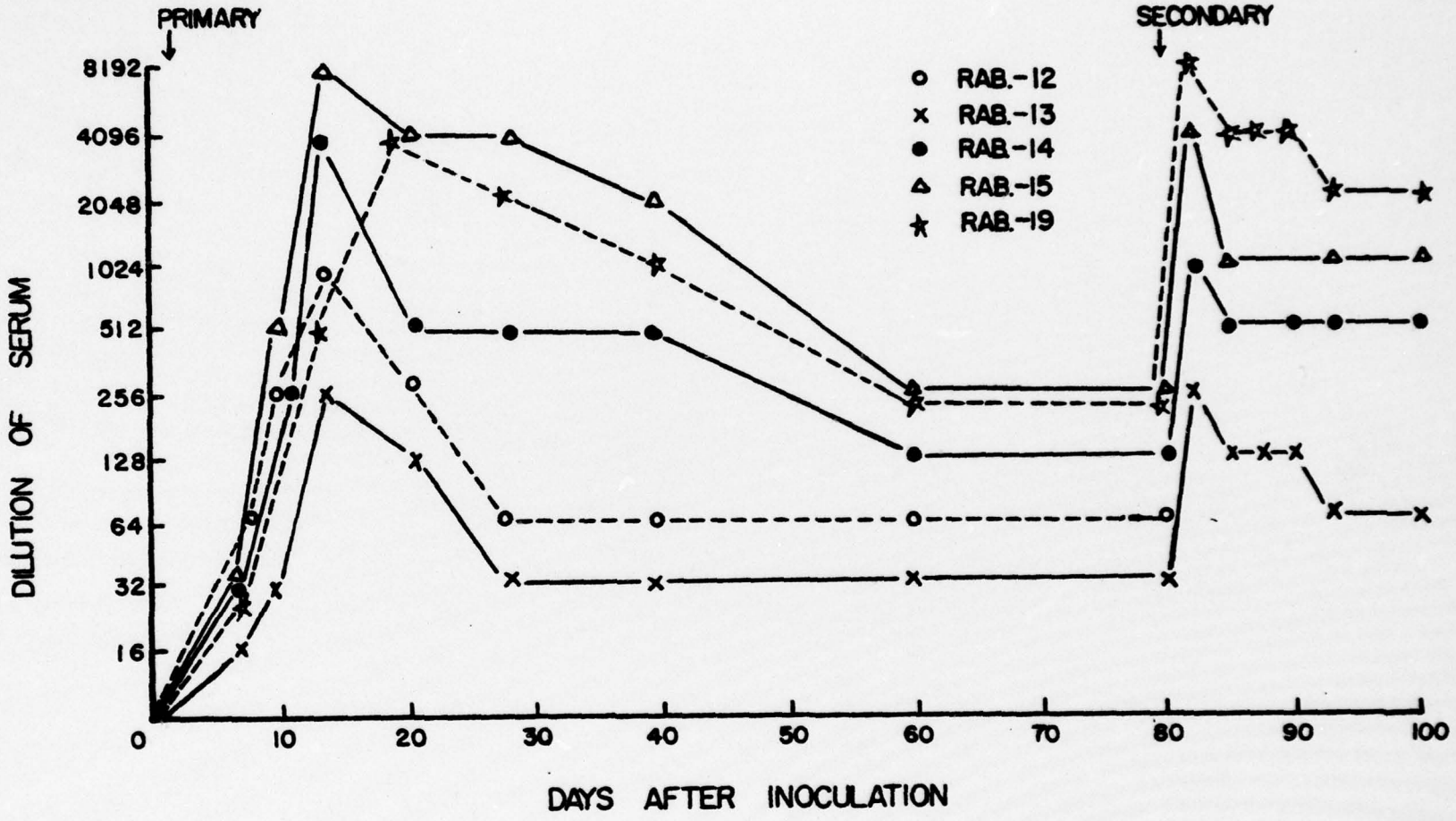

FIG. 5. ANTIBODY RESPONSES TO KHF AGENT IN RABBITS

Table 1. Hospitalized cases of Korean hemorrhagic fever patients

Year	US forces	ROK army	Korean civilian	Total
1951	827			827
1952	833			833
1953	455			455
1954	307		19	326
1955	20			20
1956	28	26		54
1957	13	21		34
1958	15	20		35
1959	79	47		126
1960	10	185		195
1961	27	341		368
1962	29	311		340
1963	11	257		268
1964	22	205	18	245
1965	99	110	2	211
1966	36	82	11	129
1967	31	86	13	130
1968	28	102	13	143
1969	9	134	8	151
1970	13	221	85	319
1971	2	358	311	671
1972	0	203	186	389
1973	0	237	241	478
1974	0	251	170	421
1975	1	370	466	837
1976	4	304	521	829
1977	7	212	288	507
Total	2,906	4,083	2,352	9,341
Fatality	5%	6.5%	8%	6.5%

Table 2. Isolation of KHF agent from wild rodents collected in endemic areas by month, 1974-1977

Species	Month and no. positives/no. tested												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
Apodemus agrarius coreae	3/46	0/19	1/64	5/40	14/74	6/71	1/52	4/27	29/80	21/122	8/72	1/29	93/696
Microtus fortis pelliceus	0/12	0/12	0/14	0/3	0/29	0/6	0/8		0/2	0/12	0/3	0/2	0/103
Crocidura lasiura	0/5		0/6	0/3	0/2	0/9	0/2		0/2	0/21	0/15	0/4	0/68
Clethrionomys rufocanus regulus	0/7	0/1	0/9	0/3	0/10	0/1				0/2	0/1		0/34
Cricetulus triton nestor			0/1		0/1	0/4	0/1		0/1	0/1			0/9
Mus musculus yamashinai	0/1						0/1		0/3	0/2	0/1		0/8
Micromys minutus ussuricus	0/1		0/1										0/2
Tamias sibiricus asiaticus					0/1								0/1

Table 3. Isolation of KHF agent from KHF patient by inoculation into Apodemus

Code of patient	Day of blood	Phase of illness	IF antibody titer	No. infected / No. inoculated
76-109	4	febrile	< 8	4/8
76-242	2	"	< 8	0/5
76-243	3	oliguric	< 8	2/7
76-253	2	febrile	< 8	0/3
76-259	3	"	< 8	0/2
76-260	5	oliguric	< 8	0/5
76-267	3	febrile	< 8	0/4
76-270	5	"	< 8	0/5
76-274	3	"	< 8	0/4
76-288	2	"	< 8	0/5
76-387	6	oliguric	< 8	0/5

$$\frac{\text{Total No. of KHF virus isolated}}{\text{Total No. of patient's sera tested}} = \frac{2}{11}$$

Table 4. Duration of infection after KHF agent inoculation into *Apodemus agrarius coreae*

Dose and route of inoculation	Days after inoculation									
	9	10	12	13	14	20	23	27	33	69
KHF agent 76/118 passage 1, 5% lung suspension intralung 0.05 ml. subcutaneous 0.1 ml.	$\frac{0}{4}$	$\frac{1}{4}$	$\frac{2}{4}$	$\frac{3}{4}$	$\frac{2}{4}$	$\frac{3}{4}$	$\frac{1}{3}$	$\frac{7}{13}$	$\frac{2}{4}$	$\frac{1}{6}$

$\frac{\text{No. positive}}{\text{No. tested}}$

Table 5. Distribution of KHF agent (76/118) in tissues of Apodemus agrarius coreae after experimental inoculation

Route and dose of inoculation	Tissue	Days after inoculation				
		9	13	20	27	33
Intrapulmonary 0.1 ml.	Lung	-	+++	++++	++	+++
	Kidney	-	-	++	-	++
	Liver			++	-	-
	Parotid gl.			+	-	-
	Submaxillary gl.			-	-	+
	Bladder			+	-	+
	Spleen	-	-	-	-	-
Subcutaneous 0.1 ml.	Lung	-	+++	++++	++++	++++
	Kidney	-	-	++	++	++
	Liver		-	+	++	+
	Parotid gl.			-	-	+
	Submaxillary gl.		-	-	+	-
	Spleen	-	-	-	-	-
Intraperitoneal 0.1 ml.	Lung	-	-	++++	-	-
	Kidney	-	-	+	-	-
	Liver			-	-	
	Parotid gl.			-		
	Spleen			-	-	-
Nasal and oral 0.1 ml.	Lung	-	+	+++	-	-
	Kidney	-		-	-	-
	Parotid gl.	-	-	-	-	
	Liver			-	-	
	Spleen		-	-	-	-

Inoculum: Supernatant of 5% infected lungs and kidneys suspension.

Table 6. Distribution of KHF agent in tissues of Apodemus agrarius coreae caught in the endemic area

Tissue	Code number of rodent, place and date of collection					
	R-76-66 Yunchun 5/6	R-76-79 Songnaeri 5/13	R-76-81 Songnaeri 5/13	R-76-98 Songnaeri 6/2	R-76-100 Songnaeri 6/2	R-76-118 Songnaeri 6/23
Lung	++++[a]	++	++++	++	+++	+++
Kidney	++	+	++	-	+	++
Parotid glands	++	+	+	+	+	+
Bladder	+	-	+	-	-	+
Liver	++	-	+	-	-	+
Submaxillary glands	+	-	+	-	-	-
Spleen	-	-	-	-	-	-
Intestine	+	-	-	-	-	-

[a] Fluorescent antigen was graded + to ++++ according to intensity and particularly the extent of reaction in tissues.

Table 7. Propagation of KHF agent (strain 76-118) in *Apodemus agrarius coreae*

Number of passage	Total days in mice	Cumulative log dilution of original inoculum	Infectivity for mice	Titration in mice ID_{50}/0.1 ml.
1	20	1.0	10/16	
2	47	2.0	7/13	
3	70	3.0	14/32	
4	91	4.0	7/28	
5	111	5.0	23/34	
6	131	6.0	3/4	
7	151	8.0	12/12	$10^{4.2}$
8	171	12.0	9/9	$10^{5.3}$
9	191	17.0	5/5	
10	211	19.0	6/6	
11	231	21.0	21/21	$10^{7.2}$
12	251	27.0	3/3	
13	271	29.0	3/3	
14	291	31.0	3/3	
15	311	33.0	3/3	
16	324	38.0	15/15	$10^{6.7}$
17	337	43.0	3/3	
18	349	45.0	5/5	
19	360	47.0	3/3	
20	372	49.0	3/3	
21	383	51.0	5/5	
22	395	53.0	3/3	
23	406	55.0	3/3	
24	417	57.0	3/3	
25	430	60.0	3/3	
26	441	63.0	3/3	

Table 8. Occurrence of immunofluorescent antibodies to KHF agent in human sera

Group	No. positive / No. tested	(%)
Clinically classified severe cases	113/116	(97.4)
Clinically suspected mild cases	11/34	(32.4)
Blood donors, Seoul	3/255	(1.2)
Out patients, all ages Seoul and Chunchun	2/204	(1.0)
Resident of endemic area, Tongduchun	6/159	(3.8)
Military personnel, Pochun	4/378	(1.1)

Table 9. Serologic relationship between KHF agent and other viral hemorrhagic fevers

Antigen	Name of antiserum	Immuno-fluorescent antibody test
Lung tissue of infected *Apodemus* with KHF agent 76/118, 6th passage	Anti-Pichinde monkey serum	-
	Anti-Tacaribe monkey serum	-
	Anti-Machupo human serum	-
	Anti-Lassa human serum	-
	Anti-LCM guinea pig serum	-
	Anti-Ebola human serum	-
	Anti-Marburg human serum	-
	Convalescent sera of KHF	+
	Convalescent sera of hemorrhagic fever with renal syndrome, Soviet Union	+
	Convalescent sera of epidemic hemorrhgic fever, Japan	+

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